

Applicants: Chong-Jin Oon et al.  
Serial No.: CPA of 09/362,394  
Filed : July 28, 1999  
Page 3

D2 (C-7 amine)-3' (SEQ ID NO:4), and 5'-(6FAM)TACGGACAGAACTGTTTTTTTTTTT (C-7 amine)-3' (SEQ ID NO:5), and the second oligonucleotide contains the mutation G to A (position 8 of the oligonucleotide, in bold) leading to change at amino acid 145 (Glycine to Arginine) of human hepatitis B virus surface antigen. There is also an inclusion of a poly-T (underlined) as a synthetic linker aiming at optimizing the subsequent hybridization reaction with target human viral DNA sequences from serum samples.--

A mark up copy of the amendments to the specification is attached hereto as **Exhibit 1**.

In the Claims

Please cancel claim 37 without prejudice or disclaimer.

Please amend claims 20, 21, 26, 29, 30, 35, 36, 39, 41, 43, 44, 46, 47, 48, 49, 50, 52, 53, 54, 57, 58, 59, 60, 61, 62, 64, 65, and 66 as follows:

D3 --20. (Amended) The oligonucleotide of claim 19, wherein the oligonucleotide comprises the sequence TACGGACGGAACT (SEQ ID NO:3).--

--21. (Amended) The oligonucleotide of claim 19, wherein the oligonucleotide comprises the sequence TACGGACGGAACTGTTTTTTTTTTT (SEQ ID NO:4).--

D4 --26. (Amended) An oligonucleotide which (1) is immobilized, (2) comprises the sequence TACGGACGGAACTGTTTTTTTTTTT (SEQ ID NO:4), (3) is linked to 6-(fluorescein-6-carboxamido) hexanoate at its 5' terminus, and (4) is

linked to a C-7 amine at its 3' terminus.--

--29. (Amended) The oligonucleotide of claim 27, wherein the oligonucleotide comprises the sequence TACGGACAGAACT (SEQ ID NO:1).--

--30. (Amended) The oligonucleotide of claim 27, wherein the oligonucleotide comprises the sequence TACGGACAGAACTGTTTTTTTTTTT (SEQ ID NO:5).--

--35. (Amended) An oligonucleotide which (1) is immobilized; (2) comprises the sequence TACGGACAGAACTGTTTTTTTTTTT (SEQ ID NO:5), (3) is linked to 6-(fluorescein-6-carboxamido) hexanoate at its 5' terminus, and (4) is linked to a C-7 amine at its 3' terminus.--

--36. (Amended) An oligonucleotide which (1) has a sequence which corresponds to a portion of a nucleic acid which encodes human hepatitis B virus surface antigen, wherein the sequence is AGGATCAACAACAACCGTA (SEQ ID NO:6), and (2) is linked at its 5' terminus to a biotin group.--

--39. (Amended) The oligonucleotide of claim 38, wherein the sequence is ATCGTCCTGGGCTTTCGCAA (SEQ ID NO:7).--

--41. (Amended) The oligonucleotide of claim 38, wherein the sequence is ATCGTCCTGGGCTTTCGCAA (SEQ ID NO:7), and the fluorescent dye is Texas red.--

--43. (Amended) The composition of claim 42, wherein (i) the first oligonucleotide has the sequence

Applicants: Chong-Jin Oon et al.  
Serial No.: CPA of 09/362,394  
Filed : July 28, 1999  
Page 5

AGGATCAACAACAACCGTA (SEQ ID NO:6); and (ii) the second oligonucleotide has the sequence ATCGTCCTGGGCTTTCGCAA (SEQ ID NO:7), and the fluorescent dye is Texas red.--

--44. (Amended) A method for identifying a human hepatitis B virus surface antigen mutant 145 in a sample which comprises:

- Q9
- (A) obtaining viral nucleic acid from a sample;
  - (B) amplifying the viral nucleic acid in a polymerase chain reaction using two primers, wherein
    - (1) one primer is a first oligonucleotide which
      - (i) has a sequence which corresponds to a portion of a nucleic acid which encodes human hepatitis B virus surface antigen, and
      - (ii) is linked at its 5' terminus to a biotin group; and
    - (2) the other primer is a second oligonucleotide which (1) has a sequence which is complementary to a nucleic acid which encodes human hepatitis B virus surface antigen, and (2) is linked at its 5' terminus to a fluorescent dye;
  - (C) obtaining, from the amplified nucleic acid, single stranded nucleic acid which comprises the fluorescent dye;
  - (D) contacting the single stranded nucleic acid which comprises the fluorescent dye to an immobilized third oligonucleotide, which oligonucleotide comprises a sequence which (i) corresponds to a portion of a human hepatitis B virus surface antigen nucleic acid, which portion comprises a mutation present in a mutant human hepatitis B

Applicants: Chong-Jin Oon et al.  
Serial No.: CPA of 09/362,394  
Filed : July 28, 1999  
Page 6

Q9 virus, (ii) is linked to a fluorescent dye at its 5' terminus; and (iii) is linked to a primary amine group at its 3' terminus, under conditions permitting hybridization between the single stranded nucleic acid which comprises the fluorescent dye and the third oligonucleotide, wherein hybridization between the single stranded nucleic acid which comprises the fluorescent dye and the immobilized third oligonucleotide identifies the sample as one containing a human hepatitis B virus surface antigen mutant 145.--

--46. (Amended) The method of claim 44, wherein the third oligonucleotide comprises the sequence TACGGACAGAACT (SEQ ID NO:1).--

--47. (Amended) The method of claim 44, wherein the third oligonucleotide comprises the sequence TACGGACAGAACTGTTTTTTTTTTT (SEQ ID NO:5).--

Q10 --48. (Amended) The method of claim 44, wherein the fluorescent dye which is linked to the third oligonucleotide is 6-(fluorescein-6-carboxamido) hexanoate.--

--49. (Amended) The method of claim 44, wherein the primary amine group which is linked to the third oligonucleotide is a C-7 amine.--

--50. (Amended) The method of claim 44, wherein the third oligonucleotide is immobilized on a solid support.--

Q11 --52. (Amended) The method of claim 44, wherein the third

oligonucleotide (1) is immobilized, (2) comprises the sequence TACGGACAGAACTGTTTTTTTTTTT (SEQ ID NO:5), (3) is linked to 6-(fluorescein-6-carboxamido) hexanoate at its 5' terminus, and (4) is linked to a C-7 amine at its 3' terminus.--

--53. (Amended) The method of claim 44, wherein the primer in step (B)(1) has the sequence AGGATCAACAACAACAGTA (SEQ ID NO:6).--

--54. (Amended) The method of claim 44, wherein the primer in step (B)(2) has the sequence ATCGTCCTGGGCTTTCGAA (SEQ ID NO:7).--

--57. (Amended) A method for identifying a wildtype human hepatitis B virus surface antigen in a sample which comprises:

- (A) obtaining viral nucleic acid from a sample;
- (B) amplifying the viral nucleic acid in a polymerase chain reaction using two primers, wherein

- (1) one primer is a first oligonucleotide which
    - (i) has a sequence which corresponds to a portion of a nucleic acid which encodes human hepatitis B virus surface antigen, and
    - (ii) is linked at its 5' terminus to a biotin group; and

- (2) the other primer is a second oligonucleotide which (1) has a sequence which is complementary to a nucleic acid which encodes human hepatitis B virus surface antigen, and (2) is linked at its 5' terminus to a fluorescent dye;

- (C) obtaining, from the amplified nucleic acid,

single stranded nucleic acid which comprises the fluorescent dye;

(D) contacting the single stranded nucleic acid which comprises the fluorescent dye to an immobilized third oligonucleotide, which oligonucleotide comprises a sequence which (1) corresponds to a portion of a wildtype human hepatitis B virus surface antigen nucleic acid, (2) is linked to a fluorescent dye at its 5' terminus; and (3) is linked to a primary amine group at its 3' terminus, under conditions permitting hybridization between the single stranded nucleic acid which comprises the fluorescent dye and the third oligonucleotide,

12 wherein hybridization between the single stranded nucleic acid which comprises the fluorescent dye and the third oligonucleotide identifies the sample as one containing a wildtype human hepatitis B virus surface antigen.--

--58. (Amended) The method of claim 57, wherein the third oligonucleotide comprises the sequence TACGGACGGAAACT (SEQ ID NO:3).--

--59. (Amended) The method of claim 57, wherein the third oligonucleotide comprises the sequence TACGGACGGAAACTGTTTTTTTTTTT (SEQ ID NO:4).--

--60. (Amended) The method of claim 57, wherein the fluorescent dye which is linked to the third oligonucleotide is 6-(fluorescein-6-carboxamido) hexanoate.--